Silencer® Select Human GPCR siRNA Library V4

Package Contents

Catalog Number	Size
4397916	0.25 nmol each siRNA

- 1.75 mL Nuclease-free Water

Storage Conditions

- Store at or below –20°C. Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.)
- 12-month shelf life

Required Materials

- RNase-free reagents
- Transfection reagent e.g. Lipofectamine® RNAiMAX

Timing

Transfection preparation: 15 minutes
Final incubation: 1–3 days

Selection Guide

siRNAs
Go online to view related products.

Product Description

- Silencer® Select siRNAs are chemically modified, 21-mer, double-stranded RNAs (dsRNAs) with third generation locked nucleic acid (LNA) chemistry for increased potency and specificity as compared to unmodified 21-mer dsRNAs (Silencer siRNA).
- This library contains 1137 unique siRNAs (0.25 nmol). Three unique, non-overlapping siRNAs are provided for each of 379 human targets. This siRNA library is supplied in 96-well plates.

Transfection Guidelines

- Handling instructions: RNA oligonucleotides are susceptible to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips.
- Transfection efficiency varies according to the cell type and transfection agent used. To optimize, determine the conditions that result in maximum gene silencing with minimal cytotoxicity. Maintain conditions across experiments, and use positive and negative controls in all plates.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

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Print Options

Library Contents and Target Information

This library contains 1137 unique siRNAs targeting each of 379 human GPCR genes*. Contents include a total of 15, 96-well plates (plates are Axygen Catalog No. PCR96FS; www.axygen.com).

- 9 plates with 88 siRNAs each
- 3 plates with 87 siRNAs each
- 3 plates with 28 siRNAs each

*A few siRNAs target more than one gene's transcript(s), due to gene families with highly homologous members or predicted genes with high homology to verified genes.

siRNA Resuspension Protocol

We recommend preparing 10 µM siRNA stock solution.

1. Briefly centrifuge the plate to ensure that the dried siRNA is at the bottom of the tube.
2. Resuspend the 0.25 nmol siRNA using 25 µL of the nuclease-free water provided for a final concentration of 10 µM.
3. (Optional) Aliquot siRNAs into daughter tubes or plates to limit the number of freeze-thaw cycles to which the siRNAs are subjected. Solutions at concentrations >2 µM can undergo up to 50 freeze-thaw cycles without significant degradation.
4. Store at or below –20°C in a non-frost-free freezer until use.

Once reconstituted in nuclease-free water, the siRNA is ready to transfect and can be used at your choice of final concentration.

RNAi Transfection Protocol

See page 2 to view guidelines for transfecting siRNAs using Lipofectamine® RNAiMAX Reagent. We recommend using 10 nM siRNA concentration as a starting point.

Reverse Transfection of RNAi

Reverse transfection is faster to perform than forward transfection and is the method of choice for high-throughput transfection. Perform reverse transfection by preparing the siRNA transfection complexes inside the wells, and then adding cells and medium. Because the cells and siRNA-reagent complexes are prepared on the same day, we recommended using 2.5× more cells than for a regular transfection.

Limited Product Warranty and Disclaimer Details

Limited Use Label Licenses

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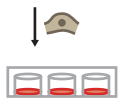
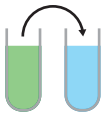
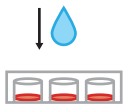
RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine® RNAiMAX.
The prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.

Timeline		Steps
Day 0	1 	**Seed cells to be 60-80% confluent at transfection**
	2 	**Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium**
Day 1	3 	**Dilute siRNA in Opti-MEM® Medium**
	4 	**Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)**
	5 	**Incubate**
	6 	**Add siRNA-lipid complex to cells**
Day 2–4	7 	**Visualize/analyze transfected cells**

Procedure Details

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Opti-MEM® Medium	25 µL	50 µL	150 µL
Lipofectamine® RNAiMAX Reagent	1.5 µL	3 µL	9 µL
Opti-MEM® Medium	25 µL	50 µL	150 µL
siRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
Diluted siRNA	25 µL	50 µL	150 µL
Diluted Lipofectamine® RNAiMAX Reagent	25 µL	50 µL	150 µL
Incubate for 5 minutes at room temperature.			
Component	**96-well**	**24-well**	**6-well**
siRNA-lipid complex per well	10 µL	50 µL	250 µL
Final siRNA used per well	1 pmol	5 pmol	25 pmol
Final Lipofectamine® RNAiMAX used per well	0.3 µL	1.5 µL	7.5 µL
Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells.			